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EXPERIMENTAL STUDIES ON MILK.*†

WITH ESPECIAL REFERENCE TO THE UNIFORMITY OF DIFFERENT GRADES OF MILK, AND THE EFFECTS OF STORAGE UPON CERTIFIED, INSPECTED, AND PASTEURIZED MILKS.

BASED UPON DAILY OBSERVATIONS OF SAMPLES COVERING A PERIOD OF TEN MONTHS.

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INDEX AND OUTLINE.

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I. CLASSIFICATION OF MILK.

The milk of our domestic animals, especially that of the cow, is one of the most important articles of food. All people are partially dependent on milk, and children, during the early period of their lives, are entirely dependent on it. It is an especially adapted food, for the carbohydrate is in solution ready for assimilation, the fat is already in emulsion, and the protein exists in such form that it is not coagulated by the heat at times necessary to preserve it and to render it innocuous. Milk readily deteriorates, can cause and carry disease, and is easily falsified. With the development of the commercial aspects of milk the dangers of harmful additions to and abstractions from milk have become as important as its necessity as a food. It is not to be wondered at that a satisfactory control of market milk should be demanded. In more recent years this control has not so much had as its object the prevention of frauds but rather has been designed to make available milk that is not harmful to the consumer. The milk

supplies of most large cities in the United States now consist of certified milk, inspected milk, raw market milk, and pasteurized milk.

Certified milk is milk obtained from healthy cows, free from tuberculosis as determined by the tuberculin test. The cows must be well fed and cared for, cleaned before milking, milked carefully and under sanitary conditions by clean milkers free from disease. This milk must be cooled and bottled soon after milking and must always be kept cold until it is delivered to the consumer. The bacterial count per cubic centimeter must be below 10,000. In some cities the percentages of butter fat and other chemical constituents are determined, while in other cities no particular attention is given to the chemical composition of certified milk. Some medical milk commissions demand a label stating the percentage of butter fat. Certified milk is generally, though not always, licensed by, and under control of, a local board appointed by a medical society.

Inspected milk is also obtained from healthy cows, free from tuberculosis. Milking, cooling, bottling, and handling must be done under sanitary conditions. Constant refrigeration too is required. The number of bacteria per cubic centimeter must not exceed 100,000.

Market milk is milk which in its raw state may or may not meet the requirements of certified and inspected milk.

Pasteurized milk is any raw milk heated for a time to a temperature above 139° F. and below the boiling point, after which it is rapidly cooled.

By far the largest amount of milk sold is market milk, either raw or pasteurized; certified milk is principally used by infants and invalids, while inspected milk is sold largely for family use. From October 1, 1910, to August 1, 1911, daily deliveries of 12 different supplies of milk from Boston were received at the laboratory. Of these 12 supplies, seven belonged to the certified class, two were inspected, and three were of milk pasteurized in bulk by the holding method. In addition during May, June, and July, 1911, inspected milk pasteurized in the sealed bottle at 150° F. for 30 minutes was received. All of these milks were examined when delivered and

after keeping them for varying periods of time at definite temperatures. All the tests available in the general laboratory were tried at these different examinations.

II. MILKS STUDIED.

CERTIFIED MILK.

No. 1. Milk from a mixed herd of about 40 cows, shipped to the city by train.

No. 7. Milk from a mixed herd of about 60 cows, hauled to the city by wagon. Only certified milk produced on this farm.

No. 8. Milk from a mixed herd of about 30 cows, shipped to the city by rail.

No. 9. Milk from a mixed herd of about 40 cows, shipped to the city by rail. Only certified milk produced on this farm.

No. 10. Milk from a mixed herd of about 40 cows, contained over $4\frac{1}{2}$ per cent of fat. Shipped to the city by rail. Only certified milk produced on this farm.

No. 11. Milk from a mixed herd of about 40 cows, shipped to the city by rail.

No. 14. Milk from a mixed herd of about 90 cows, hauled to the city by wagon. Only certified milk produced on this farm.

INSPECTED MILK.

No. 4. Supply made up of milk from a considerable number of farms, well inspected and controlled. Shipped to the city by rail.

No. 12. Milk from one farm, shipped to the city by rail.

PASTEURIZED MILK.

No. 2. Milk from tuberculin tested cows on several farms, pasteurized at 145° F. for 20 minutes by the holding device. Pasteurized from 12 to 24 hours before delivery.

No. 3. Market milk pasteurized at 145° F. for 20 minutes by the holding device. Pasteurized 12 to 24 hours before delivery.

No. 13. Market milk pasteurized at 145° F. for 20 minutes by the holding device. Pasteurized 12 to 24 hours before delivery.

No. $4\frac{1}{2}$. Inspected milk, pasteurized in the sealed bottle at 150° F. for 30 minutes under official supervision. Pasteurized 20 to 24 hours before delivery.

III. CONDITION IN WHICH MILK WAS DELIVERED.

All deliveries except of Nos. 4 and $4\frac{1}{2}$ were made in the morning, and usually were received in good condition. The drivers of the wagons were generally responsible, bright young men, and all but one were interested in delivering good milk. Milk was always received well refrigerated, but during the winter every supply except No. 4, which was delivered in the afternoon, was frozen at some time. This applied to certified as well as market milks. The greatest irregularity found was in the dating of certificates issued by the milk commissions. It not infrequently happened that certificates one to several months old were used and on investigation it was learned that the commissions had not supplied new certificates or had not prepared enough for the month. This condition did not occur as frequently after the summer of 1911 as before that time. Sour milk or curdled milk was never delivered. Pasteurized milk or any milk that has gone through a clarifier is generally somewhat more cream-colored than certified milk. Visible dirt was never found in the milk furnished, although at times the bottles were chipped, soiled from the ice, and not as carefully capped as they should be.

EXAMINATION OF MILK AS DELIVERED AND AFTER STORAGE.

Examination of milk is made to determine its food value and ingredients, the presence or absence of disease-producing elements, the presence or absence of the milk ferments, and to detect if there is any deterioration due to age, heat, or other processes. Methods of determining these facts differ and are numerous. In this investigation only those tests were made that deal with the hygienic or sanitary value of milk; furthermore only such tests were used as give results that may be easily and readily interpreted, for these, after all, are the ones that are of practical assistance, because a new supply of milk must be available daily. Officials in most cities acknowledge that there is no test immediately available by which

they can prevent the sale and consumption of milk that is not good —excepting, possibly, the temperature test, or the dirt test.

IV. SEDIMENTATION TESTS.

These tests are used principally to determine the presence of dirt and body cells, especially leukocytes. To detect these a number of methods have been devised. Dirt is frequently tested for by filtration through cotton, or by allowing large amounts of milk to stand in conical glasses. Usually garget is determined by straining through cloth or by smears made directly from milk[1] or from the sediment of centrifugalized milk. It was found that in the milks delivered, dirt was seldom demonstrable by filtration through cotton or by allowing it to stand, and that the making and examinations of smears requires much time and gives results which are not universally accepted.

The use of the Tromsdorff tube, which holds about 14 c.c. and has a small tube at the bottom, is satisfactory and gives reliable results. In these tubes 10 c.c. of milk is put and centrifugalized for 15 minutes. According to Auzinger[2] a sediment over 1.0 is unfavorable, and milk for infant feeding, from a mixed herd, should not be above one-fourth, and from a single cow not above one-half. Repeated microscopic examinations of the sediment in a Tromsdorff tube shows that a yellow sediment consists of cells, a white sediment is largely casein, and a gray sediment is due to dirt. According to Rühm leukocytes appear in milk at the beginning and end of lactation, due to accumulations of milk as the result of incomplete milking, from leukocytosis, as in fevers, etc., and in old milkers. Without going into the argument of leukocytes in milk, the results of the frequent determinations made with the Tromsdorff tube are given in Table 1.

From this table it is seen that relatively few samples were free from sediment. Market milk contains dirt much more frequently than certified or inspected milks. This is to be expected because market milk is made up of a mixture of milk from a large number of ordinary farms. Cells on the other hand occur more frequently

[1] Prescott and Breed, *Jour. Infect. Dis.*, 1910, 7, p. 632.

[2] *Ztschr. f. Fl. u. Milch-Hyg.*, 1910, 20, pp. 368, 400.

in certified and inspected milks than in the pasteurized market milk. This is undoubtedly due to the fact that the clarifier removes body cells more efficiently than finely divided dirt. Few of the

TABLE 1.

SHOWING PERCENTAGE OF SAMPLES CONTAINING BODY CELLS AND DIRT, AND HIGHEST AND LOWEST TROMSDORFF READINGS.

Supply	No.	Body Cells			Dirt			No Sediment
		Percentage	Largest Amount	Smallest Amount	Percentage	Largest Amount	Smallest Amount	Percentage
Certified	1	43	2/3	0.0	57	Trace	0	0
	7	86	1 1/4	0.0	14	Trace	0	0
	8	62	1/2	0.0	26	1/4	0	12
	9	50	1/4	0.0	50	1/8	0	0
	10	62	1/2	0.0	38	1/8	0.0	0
	11	100	3/8	Trace	0	0	...	0
	14	75	1/2	0	25	1/4	0.0	0
	Average	68	1 1/4	0	30	1/4	0.0	2
Insp.	4	80	3/4	0	20	Trace	0.0	0
	12	75	3/8	0.0	12.2	1/8	0.0	12.5
	Average	77	3/8	0.0	16	1/8	0.0	7.5
Pasteurized	2	55.5	1/4	0.0	44.5	1/4	0.0	0.0
	3	62.5	1/3	0.0	27.5	1/4	0.0	.0
	13	28.5	1/8	0.0	71.5	1/8	0.0	0.0
	4 1/2	78	3/8	0	22	1/8	0.0	0.0
	Average, 2, 3, 13	50	1/3	0.0	50	1/4	0.0	0.0

samples, however, showed sediment to the mark ½, a large number only containing a trace. In only one sample was the sediment sufficient to exceed the mark 1.0, and this was due to body cells as confirmed by examination of a suitably stained slide. All of our better classes of milk contain little sediment.

V. BACTERIOLOGICAL EXAMINATIONS.

1. METHODS AND TECHNIC.

The bacteria in milk are of importance both from health and from commercial standpoints. As far as health is concerned it is especially important to know whether milk contains pathogenic bacteria. To determine this, however, requires time, labor, and expense, so that it is not feasible to examine all milk for specific microorganisms. Of the available routine examinations, the determination of the number of bacteria per cubic centimeter gives the most valuable information, because infection of the udder, dirty methods, poor refrigeration, and age all lead to higher bacterial

counts. In addition, however, to determining the bacterial count it is of importance to know the action the bacteria will have on the milk as well as the effect storage at certain temperatures will have on these microorganisms. For this reason determinations of the total number and of fermenting and peptonizing organisms in one cubic centimeter, and of the presence of organisms liquefying gelatin, producing indol and hydrogen sulfid were made on the different milks as received, and after varying periods of storage at definite temperatures. The number of bacteria per cubic centimeter was determined by the standard method as outlined by a committee of the American Public Health Association[1] in 1908. The number of organisms fermenting lactose was determined by adding 1 per cent of lactose to neutral litmus agar. To determine the number of bacteria breaking up the protein of milk, one cubic centimeter of sterile skimmed milk was put into the petri dish, after which the proper dilution of milk was added, and finally molten sugar-free agar was poured in. After 48 hours of incubation, a dilute solution of acetic acid was run over the plate, then the number of proteolytic organisms producing colonies surrounded by a clear zone was determined. This is essentially the method of Hastings.[2]

The amount of gas and the gas formula were determined in lactose broth in Smith fermentation tubes, according to the usual method. The fermentation tubes were inoculated with 1 c.c. of milk and incubated at 37° C. for 48 hours.

Liquefaction of gelatin was determined in sugar-free gelatin. One cubic centimeter of milk was put on the hardened gelatin, the height of gelatin in the tube being marked. After this a straight stab was made through the medium with the platinum needle. The stab was made so as to insure inoculation because the cream on rising carries with it so many bacteria that all the liquefiers may be carried away from the surface of the gelatin. These tubes were incubated at room temperature for 10 days.

Hydrogen sulfid and indol production were tested by inoculation of Dunham's solution with 0.1 c.c. of milk in a test tube containing a strip of filter paper saturated in lead acetate held by the cotton plug. The tubes were incubated for 10 days at 37° C. when

[1] *Am. Jour. Pub., Hyg.*, 1908, 18, p. 425.

[2] *Centralbl. f. Bakt. u. Parasit.*, 1902, 10 ,p. 384, and 1904, 12, p. 590.

the readings for hydrogen sulfid were made. After this indol was tested for in the regular way.

2. BACTERIAL COUNTS.

The total number of peptonizing and fermenting bacteria per cubic centimeter was determined at frequent intervals, and it seems sufficient to give the average and highest counts obtained.

BACTERIAL COUNTS OF MILKS AS RECEIVED.

The following table shows the results obtained in the examinations of certified, inspected, and pasteurized milk as it is delivered.

TABLE 2.

SHOWING AVERAGE, HIGHEST, AND LOWEST BACTERIAL COUNTS, AND MONTHS IN WHICH HIGHEST AND LOWEST COUNTS OCCURRED.

CERTIFIED MILK.

Number	Average Count	Highest Count	Lowest Count	Percentage over 10,000	Percentage 5,000–10,000	Percentage 1,000–5,000	Percentage less than 1,000	Time of Highest	Time of Lowest
1	3,756	10,500	800	6	35	47	12	January	November
7	6,650	27,000	1,800	31	38	31	..	November, 1910	August, 1911
8	8,935	24,000	1,400	36	21	43	..	November	May, June
9	20,650	51,000	3,500	75	..	25	..	November	February, June
10	13,900	52,000	900	56	18	18	8	November	May
11	3,543	14,000	600	7	15	57	21	March	June
14	3,470	7,100	800	0	9	73	18	November	August

INSPECTED MILK.

Number	Average Count	Highest Count	Lowest Count	Percentage 50,000–100,000	Percentage 10,000–50,000	Percentage 5,000–10,000	Percentage 1,000–5,000	Percentage less than 1,000	Time of Highest	Time of Lowest
4...	33,610	52,000	12,500	8	92	..	..	....	November	March
12...	11,545	99,000	1,300	21	28	23	28	....	June	March

PASTEURIZED MARKET MILK.

Number	Average Count	Highest Count	Lowest Count	Percentage over 1,000,000	Percentage 500,000–1,000,000	Percentage 50,000–500,000	Percentage 10,000–50,000	Percentage 5,000–10,000	Percentage 1,000–5,000	Percentage below 1,000	Time of Highest	Time of Lowest
2...	310,250	840,000	6,000	..	40	27	20	13	..	..	August	November
3...	285,875	1,640,000	16,000	..	53	31	16	..	..	..	February	November
13...	916,333	1,560,000	4,000	..	31	46	8	15	..	..	March	December
4½..	4,020	9,700	200	..	..	..	0	30	40	30	July	May

From Table 2 it will be seen that while the average bacterial count for certified milks was below the 10,000 maximum count allowed, still the producers of certified milks, numbers 7, 8, 9, and 10, had considerable difficulty in keeping below the maximum. When, however, the producer has properly adjusted conditions, he can produce milk of low count rather consistently as is the case with milks 1, 11, and 14. Of the inspected milks, 4, which is made up of milk from a number of farms, while never containing less than 12,500 bacteria per cubic centimeter, never contained above 50,000 and gave an average of 33,610. Inspected milk 12 had a bacterial content as low as 1,300, but also as high as 99,000, showing that at times there was a break in technic. Market milks 2, 3, and 13 contained rather large numbers of bacteria per cubic centimeter, for pasteurized milk. That pasteurization or storage after pasteurization must have been faulty is shown by the low counts observed at times.

In comparison with 2, 3, and 13, supply $4\frac{1}{2}$, which was heated in the sealed bottle to 150° F. for 30 minutes, never contained more than 10,000 bacteria per cubic centimeter, and went as low as 200 per cubic centimeter.

It is evident from these examinations and from observation at the place of production and distribution, that generally it is comparatively easy to produce milk of low bacterial count but that the greatest care must constantly be exercised if occasional high counts are to be avoided.

The time of the year when the higher counts occur varies. The better milks generally have highest counts in the fall and winter. Especially is this true of certified milks, while the general market milk is likely to show the highest counts in the spring and summer.

Investigation of the causes of the higher counts in certified milks during the fall seems to indicate that the necessity for carrying milk to the distant milk-house through the cold air makes the milkers loath to wash their hands before milking each cow, and that winter weather generally interferes with the rigid technic of certified farms. The higher counts in market milk during the summer are probably due to more pressing work on the farm, dust and multiplication of bacteria occurring as a result of poor refrigeration,

especially in the spring when winter methods are no longer efficient and summer refrigeration has not yet been well started.

BACTERIAL COUNTS AFTER STORAGE FOR VARYING PERIODS OF TIME AT DEFINITE TEMPERATURES.

Milk, when it is offered for sale, has been exposed to storage at various degrees of temperature. While certified and inspected milks are usually kept cold from the time of production to the time of sale, ordinary market milk, which forms by far the largest part of all milk consumed, frequently is not kept cold at the farm and during transportation.

With the institution of pasteurization, refrigeration before pasteurization is no longer deemed essential. In our cities very little milk is delivered to the consumer within 24 hours after production, a large portion of the pasteurized market milk is 48 hours old, and most milk is not consumed until it is 48 hours to 72 hours old. With the institution of efficient refrigeration some milk is stored for 10 to 30 days. It is thus seen that milk is held for a time at various temperatures, and to determine the bacterial multiplication the various milks studied were held at 37° C., room temperature, the ordinary ice-box temperature (45 to 55° F.), and at a temperature just above freezing. It was found that milk of all grades after storage at 37° C. and at the room temperature showed most variable rates of bacterial increase. Furthermore, the number of bacteria per cubic centimeter varied a great deal when coagulation occurred. After 12 hours of incubation at 37° C., certified, inspected, and pasteurized milks contained from 100 to 20,000 times the original number of bacteria, while at room temperature the rate of multiplication varied from 100 to 30,000 times the original number in 24 hours. At the temperature of the ice-box the number of bacteria for the first 24 hours varied from 20 to 200 times the original number, after 72 hours from 20 to 500 times the original number. When these milks were stored at a temperature just above freezing, little or no multiplication was observed during the first seven days, pasteurized and inspected milks showed more increase than certified milk, but after the 10th day the bacteria generally multiplied very rapidly in all milks. Realizing that in practice milk that is stored is generally not mixed until it is to be used, that the cream

on rising carries with it most of the bacteria, that when milk and cream have separated and are then mixed not nearly as large a proportion of the bacteria rise to the top, and that the rate of multiplication in cream and skimmed milk may be different, counts were made of milks that had been mixed several times as well as of milks that had not been mixed. The increase in bacteria at 1.0° C. is shown in the following table:

TABLE 3.

SHOWING AVERAGE INCREASE IN BACTERIA WHEN MILK IS STORED AT A TEMPERATURE JUST ABOVE FREEZING. FIGURES REPRESENT MULTIPLE OF ORIGINAL NUMBER PRESENT.

	7 DAYS' STORAGE	10 DAYS' STORAGE	15 DAYS' STORAGE		25 DAYS' STORAGE	
	Mixed 2 Times	Mixed 2 Times	Mixed 3 Times	Mixed Only after 15 Days	Mixed 4 Times	Mixed Only at End of 25 Days
Certified........	35	640	4,250	1,800*	12,680	8,680*
Inspected........	240	3,000	5,800	1,300	52,000	1,560
2, 3, and 13......	344	4,450	114,20	420	72,420	920

* Multiple determination based on average number of bacteria per c.c., as shown in Table 2.

BACTERIA IN CREAM AND SKIMMED MILK.

When cream is allowed to rise on milk it carries with it a considerable portion of the bacteria present in milk. Knox and Schorer[1] found that after milk has remained in a gravity separator packed in ice over night nearly all the bacteria are to be found in the cream layer; Anderson[2] states that his studies show that top milk, such as is advised for use in preparing formulae for infant feeding, contains from 10 to 500 times as many bacteria per cubic centimeter as the mixed milk, from which the cream is obtained. In 26 samples of milk Anderson found that the gravity cream contained about four times as many bacteria as the sediment layer and about one-third as many as the whole milk. This, however, varies with the quality and age of the milk. Milk of high bacterial count well refrigerated while the cream is separating yields cream containing several thousand times as many bacteria as the skimmed milk, while the cream rising on milk of low bacterial content may contain only 10 to 15 times as many as the skimmed milk. The

[1] *Arch. Ped.*, 1907, 24, p. 516.

[2] *Jour. Infect. Dis.*, 1909, 6, p. 392; *Bull. Hyg. Lab.*, 1909, 56, p. 739.

same milk separated into cream and skimmed milk by gravity separation, as well as by centrifugalization in the test tube, yields cream which contains a relatively larger portion of the bacteria than does cream separated by the commercial cream separator. This is shown in the following test: the number of bacteria per cubic centimeter in the whole milk was 9,800; the gravity cream which rose in 12 hours contained 35,000 and the skimmed milk 610; cream on centrifugalization in the test tube contained 22,000, the skimmed milk 1,550; 16 per cent separator cream contained 15,500 and the skimmed milk 5,200.

To determine whether bacterial multiplication is more rapid in the cream or skimmed milk, resulting from gravity separation, whole milk was stored at 1.0° C. for 14 days and 25 days. At the time of examination the milk bottle was removed from the refrigerator and 1 c.c. of cream taken out and plated; after this a small amount of the skimmed milk was taken from the bottle by means of a sterile siphon and from this plates were made, and finally all of the remaining milk was well mixed by shaking and rolling the bottle, and from this mixed milk plates were made. There was considerable variation in the number of bacteria in the different portions after long storage. The average of these results is given in the following table:

TABLE 4.

SHOWING AVERAGE RELATIONS OF NUMBER OF BACTERIA AFTER STORAGE AT 1° C., IN CREAM AND MILK COMPARED TO THE MIXED MILK.

	Storage for 14 Days			Storage for 25 Days		
	Cream to Whole Milk	Skim Milk to Whole Milk	Cream to Skim Milk	Cream to Whole Milk	Skim Milk to Whole Milk	Cream to Skim Milk
Certified	80:1	1:1200	96,000:1	460:1	1:190	87,400:1
Market pasteurized	250:1	1:270	67,500:1	39:1	1:4	156:1

Not enough examinations of inspected milk were made to warrant a fair average for comparison.

No definite conclusions on rapidity of bacterial growth in cream and skim milk can be made from these observations, as not enough examinations were made on the fresh cream and skimmed milk to offer figures for comparison. However, it will be seen that the

bacterial content after long storage was more nearly the same in the cream and skimmed milk of pasteurized market milk than in that of certified milk. Whether this is due to pasteurization or mechanical mixing as occurs in clarifiers and heaters has not been determined.

3. BACTERIA PRODUCING PEPTONIZATION AND FERMENTATION.

Many kinds of bacteria occur in milk but among these the acid-forming and -digesting species abound. The flora in milk is largely dependent on environmental conditions such as temperature, moisture, amount and character of food, the relations between the microorganisms present, and relative number of one species to the numbers of other species. Under ordinary circumstances the acid-producing organisms play the principal rôle, but under a number of conditions the putrefactive bacteria may increase sufficiently to prove their characteristic putrefactive products.

Acid formation in milk results from the decomposition of the lactose, and if it is removed as has been done by a number of investigators, the general results of Babcock, Russell, Vivian, and Hastings[1] are obtained. Under these conditions more liquefying organisms are present and putrefaction changes occur. The putrefactive organisms grow better at low temperatures than do the acid-forming ones; therefore at low temperature of storage the acid-forming organisms are held in check. Ravenel, Hastings, and Hammer[2] have found that at 0° C. there is an increase in bacteria as well as an increase in the percentage of soluble nitrogen.

In pasteurization it is generally claimed that the acid-producing organisms are destroyed, while the spore-bearing, putrefactive types survive. Ravenel and Hastings[3] believe that the practical effects of cold storage are identical with pasteurization in this respect. Ayers and Johnson[4] have shown that acid-producing organisms are abundant in milk after commercial pasteurization. Most of the conclusions in regard to pasteurization are based on pasteurization at high temperatures and on laboratory tests made in a test tube or flask. Such pasteurization, however, does not

[1] *Wis. Agric. Exp. Sta.*, 1901, *18th Ann. Rept.*, p. 157.

[2] *Jour. Infect. Dis.*, 1910, 7, p. 38.

[3] *Ibid.*

[4] *Pub. Bureau of An. Ind.*, 1910, Bull. 126.

give the same results as commercial pasteurization at 140 to 145° F. in the generally used system of holding tanks, followed by cooling and bottling by means of machinery.

The predominance of acid-forming bacteria in market milk is probably due to the disproportion in numbers at the time of its production. The acid-producing bacteria in milk come largely from manure, while putrefactive types are introduced from the air, utensils, etc. With the improvement of technic in the production and distribution of milk, contamination has been reduced. This is especially true in regard to certified milk. In this grade of milk there is very little fecal contamination, so that the acid-forming organisms are largely eliminated. Protein-digesting bacteria, on the other hand, are not as efficiently eliminated because many of the groups of bacteria are not destroyed by the live steam resorted to for sterilization of pails, cans, bottles, and other apparatus necessary for cooling and bottling milk. For these reasons, even though certified milk contains fewer bacteria per cubic centimeter, the proportion of putrefying organisms to acid-forming species is higher in certified milk than in the common milks.

The importance of acid-forming and protein-digesting bacteria in milk being established, determinations of their presence and their

TABLE 5.

SHOWING PERCENTAGES OF ACID-FORMING AND PROTEIN-DIGESTING BACTERIA IN MILKS AS DELIVERED.

SUPPLY	NO.	PERCENTAGE OF LACTOSE FERMENTERS			PERCENTAGE OF PEPTONIZING SPECIES		
		Average	Highest	Lowest	Average	Highest	Lowest
CERTIFIED	1.........	26.	70	0	40.8	60	15
	7.........	33.7	66	8	31.2	50	12
	8.........	58.0	80	30	16.0	20	8
	9.........	37.2	70	5	11.5	30	0
	10.........	31.4	50	14	15.8	40	0
	11.........	39.0	85	14	38.1	90	16
	14.........	33.2	70	10	43.2	80	70
INSP.	4.........	20.1	48	0	16.3	28	1
	12.........	45.	80	40	18.0	50	5
PASTEURIZED	2.........	34.1	80	0	13.7	60	0
	3.........	29.3	70	8	42.3	50	20
	13.........	38.3	50	20	38.0	80	15
	4½........	29.6	70	0	18.6	50	0

relative proportions were made when the milks were delivered and after storage at different temperatures for varying periods of time.

To do this, lactose litmus agar and skimmed milk agar plates, sugar-free gelatin, sugar-free broth, and lactose litmus fermentation tubes were inoculated as has already been described (Table 5).

TABLE *6a*.

SHOWING PERCENTAGE OF ACID-FORMING BACTERIA IN THE DIFFERENT GRADES OF MILK AS DELIVERED AND AFTER STORAGE.

	MILKS AS RECEIVED			37° C.		ROOM TEMP.			ICE-BOX TEMP.			1° C.			
	Average	Highest	Lowest	12 Hrs.	Coagulation	24 Hrs.	48 Hrs.	Coagulation	24 Hrs.	72 Hrs.	Coagulation	7 Days	15 Days	25 Days	Physically Changed
Certified ...	35.6	80	0	26.5	47.0	28	27.0	46.5	29	33	47.4	13	33	30.0	24.5
Inspected ..	32.5	80	0	24.0	48.2	10	10.0	14.0	25	25	27.5	13	40	21.0	27.0
Pasteurized, 2, 3, 13 ..	33.2	80	0	10.0	38.0	20	8.5	14.0	21	15	37.0	23	30	48.2	28.0
4½	29.6	70	0	20.0	31.0	18	....	10.0	6	7	10.0	50	42	25.0	....

TABLE *6b*.

SHOWING PERCENTAGE OF PROTEIN-DIGESTING BACTERIA IN THE DIFFERENT GRADES OF MILK AS DELIVERED AND AFTER STORAGE.

	MILKS AS RECEIVED			37° C.		ROOM TEMP.			ICE-BOX TEMP.			1° C.			
	Average	Highest	Lowest	12 Hrs.	Coagulation	24 Hrs.	48 Hrs.	Coagulation	24 Hrs.	72 Hrs.	Coagulation	7 Days	15 Days	25 Days	Physically Changed
Certified....	29.6	90	0	17.5	22.5	11	12.1	18	21	25	12.5	22.3	24.1	32.2	40
Inspected...	16.9	50	1	....	....	10	....	..	8	5	10.0	11.0	17.5	12.0	50
Pasteurized, 2, 3, 13...	32.0	80	0	....	....	18	7.0	..	21	15	7.5	22.5	40.0	30.0	47
4½	18.6	50	0	....	....	..	....	..	3	..	....	16.0	30.0	....	..

From Tables *6a* and *6b* it will be seen that of the bacteria in good milks at the time of delivery about 30 per cent are fermenters, that the peptonizers constitute 20 per cent of the total number in inspected milks and 30 per cent in certified and pasteurized milks. At the higher temperatures the percentage of fermenting organisms increases more rapidly and markedly in certified milk than in inspected pasteurized milks, but at the lower temperatures the percentage of acid-producing organisms is higher in pasteurized milk than in certified milk.

The protein-digesting bacteria increase in proportion only at very low temperatures, the increase being about the same in all grades of milk.

One series of determinations was made on the percentages of acid-forming and protein-digesting types in the gravity cream and skimmed milk stored for 25 days at 1° C. The results are shown in the following table:

TABLE 6c.

SHOWING PERCENTAGE OF ACID-FORMING AND PROTEIN-DIGESTING BACTERIA IN CREAM AND SKIMMED MILK WHEN MILK WAS STORED WITHOUT MIXING FOR 25 DAYS AT A TEMPERATURE JUST ABOVE FREEZING.

	WHOLE MILK		CREAM		SKIMMED MILK	
	Fermenters	Peptonizers	Fermenters	Peptonizers	Fermenters	Peptonizers
	Percentage	Percentage	Percentage	Percentage	Percentage	Percentage
Certified	43	25	30	31	61	20
Inspected	50	25	15	30	40	10
Pasteurized, 2, 3, 13	35	17	33	43	30	3

While not many tests were made, only one quart of each supply, the results found are suggestive. Apparently the skimmed milk contains the larger percentage of acid-forming types and the cream the larger proportion of the protein-digesting bacteria. This may account for the early appearance of peptonization immediately under the cream line.

FERMENTATION OF LACTOSE IN FERMENTATION TUBES.

While by means of lactose litmus agar plates the percentage of acid-forming organisms can be obtained, it must be realized that to make plates for numerical determinations very small amounts of milk must at times be used. In milk containing only a few fermenting organisms these organisms may be lost because of dilution. To overcome this difficulty lactose litmus fermentation tubes were inoculated with 1 c.c. of milk. The results are shown in the following table:

TABLE 7.

SHOWING PERCENTAGE OF SAMPLES 1 C.C. OF WHICH CONTAINED AT THE TIME OF DELIVERY ORGANISMS PRODUCING GAS IN LACTOSE LITMUS FERMENTATION TUBES.

	CERTIFIED							INSPECTED		PASTEURIZED				SUMMARY		
	1	7	8	9	10	11	14	4	12	2	3	13	4½	Certified	Inspected	Pasteurized Nos. 2, 3, 13
Percentage gas+	40	60	50	30	10	0	100	50	60	10	100	60	10	41.43	55	84
Percentage gas 1–10	40	20	0	30	0	0	20	40	60	30	50	40	0	16.66	50	35
Percentage gas 10+	0	40	50	0	10	0	80	10	0	70	50	20	10	25.82	5	49

The gas formula varied markedly, equal percentages of carbon dioxid and hydrogen occurring when the percentage of gas was high. On storage of the different milks, there was little change in the percentage of samples fermenting lactose with gas formation, except that storage at 37° C. slightly increased the percentage producing gas, and storage at 1° C. decreased it somewhat.

LIQUEFACTION OF GELATIN AND PRODUCTION OF HYDROGEN SULFID AND INDOL.

Breaking up of gelatin is closely allied to protein decomposition and the production of hydrogen sulfid and indol is regarded as positive evidence of putrefaction. Gelatin and sugar-free broth were inoculated with milk when it was received and after storage at different temperatures, so that the presence of protein-digesting organisms might be determined. The results are shown in the following table:

TABLE 8.

SHOWING PERCENTAGE OF MILKS CONTAINING AT THE TIME OF DELIVERY ORGANISMS LIQUEFYING GELATIN AND PRODUCING HYDROGEN SULFID AND INDOL.

	Certified							Inspected		Pasteurized				Summary		
	1	7	8	9	10	11	14	4	12	2	3	13	4½	Certified	Inspected	Pasteurized 2, 3, 13
Gelatin	90	79	84	90	89	58	58	90	88	100	79	79	100	78.0	88	90.0
H_2S	40	50	63	40	60	63	70	50	80	100	50	70	30	54.4	65	73.3
Indol	21	42	42	44	33	0	50	100	88	89	89	100	0	30.3	94	93.0

From this table it will be seen that a large proportion of all milks contained organisms liquefying gelatin and that most samples of inspected and pasteurized milks contained bacteria producing indol, while a small percentage of samples of certified milk contained organisms producing indol. Bacteria producing hydrogen sulfid were found most frequently in pasteurized milks.

On storage at 37° C. the liquefiers of gelatin were crowded out or killed so that the organisms in 1 c.c. of milk no longer produce liquefaction. At room and ice-box temperatures the frequency of these organisms was little affected. When, however, milk was stored for seven to 25 days at 1° C. and then kept in the ice-box for 48 hours, gelatin was liquefied by the bacteria in 1 c.c. of all the supplies.

Organisms producing indol or hydrogen sulfid are little influenced by storage. Generally, however, hydrogen sulfid and indol are more frequently produced by the organisms in 1 c.c. of milk when the milk has been stored at the lower temperatures.

VI. REACTION OF MILK.

1. METHODS AND TECHNIC.

Milk when it is first drawn from the healthy udder of the cow has an acidity which is hardly perceptible; after a very short time the acidity may be readily detected and determined, and usually as milk gets older the acidity increases, gradually causing coagulation.

The acidity of fresh milk as determined with phenolphthalein as an indicator is due principally to the presence of carbonic acid, acid phosphates, ash, and casein. The increase in acidity which appears a very short time after milking is variously ascribed to absorption of carbonic acid, acid phosphates, and calcium compounds. The acidity that develops later is due primarily to the change of lactose into lactic acid, and results from bacterial action, this being dependent on the kinds of bacteria present, their number, and the temperature at which the milk is kept. The change known as coagulation is the result of precipitation of caseinogen by the combined action of acid and calcium salts. The acid is usually lactic and can be titrated, though coagulation also occurs as a result of the formation of amido-acids and possibly also from other causes.[1] Coagulation is regarded as a safeguard because when it occurs it is a visible sign of advancing decomposition of milk.

Acidity determinations of milk are usually made by titrating with a solution of NaOH, phenolphthalein being used as the indicator. These titrations are recorded in different ways: in this country the calculations are made as lactic acid, 1 c.c.of N/1 NaOH neutralizing 0.02 gm. of lactic acid; or also in degrees of acidity, which means number of c.c. of N/10 NaOH per 100 c.c. of milk. In Germany degrees of acidity mean number of c.c. N/4 NaOH per 100 c.c. of milk. Inasmuch as this latter standard was used, all results here indicated will be given in number of c.c. of N/4 NaOH per 100

[1] Babcock and Russell, *Wis. Agric. Exp. Sta.*, 1897, *14th Ann. Rept.*, p. 161, and 1899, *16th Ann. Rept.*, p. 157.

c.c. of milk. For transposition purposes, the following equivalents are given:

1 degree (U.S.) of acidity equals 0.009 per cent lactic acid.

1 degree (German) of acidity equals 0.0225 per cent lactic acid.

1 degree (German) of acidity equals 2.50 per cent (U.S.) acidity.

According to the different observers the acidity of normal fresh milk varies from 4.8 to 8.0 degrees. According to Richmond[1] on boiling milk coagulation occurs if the acidity is above 10.0°; there is a sour taste at 17.6°, and natural curdling occurs at room temperature if the acidity is above 34.0°.

In the work during the past year acidity determinations were made with various objects in view, especially, however, to determine acidity acquired by different grades and classes of milk when kept at different temperatures for varying periods of time, and to learn whether acidity determinations can be used in the establishments of standards for milks.

2. ACIDITY OF DIFFERENT GRADES AND CLASSES OF MILK, WHEN FRESH AND AFTER KEEPING AT DIFFERENT TEMPERATURES FOR VARYING PERIODS OF TIME.

A. FRESH MILK.

Fresh milk is variously reported as having an acidity from 4.8 to 8.0 degrees. Auzinger states that fresh milk from one cow has an acidity of 6.5 to 8.0 degrees, and that the degree of acidity of fresh milk may give valuable information in regard to the cow from which it is obtained:

If the degree of acidity is less than 5.0° suspect mastitis.

If the degree of acidity is between 5.0–6.5° suspect old milkers.

If the degree of acidity is between 8.0–9.0° suspect first or second week after parturition.

If the degree of acidity is above 9.0° suspect colostrum or pathological milk.

Rühm states that milk from a tuberculous udder has an acidity of less than 7°. Hoyberg[2] has devised a method for determining inflammatory processes in the udder of the cow based on the alkaline reaction of serum exuded in inflammatory processes, using rosolic acid in alcoholic solution as an indicator. He found that the decreased acid reaction is not proportionate to number of bacteria

[1] *Analyst*, 1900, 25, p. 116.

[2] *Ztschr. f. Fl. u. Milch-Hyg.*, 1911, 21, p. 133.

or leukocytes present in freshly drawn milk, but is proportionate to the exudation of serum.

a) *Reaction of freshly drawn milk.*—A number of determinations of acidity of milk within 15 minutes after drawing were made. Samples were taken from one quarter of the udder at the beginning, middle, and end of milking. The average results obtained were 7.0° of acidity at the beginning, 5.8° in the middle, and 6.6° at the end.

Likewise experiments were tried to test out the method of Hoyberg. His reagent is made as follows: 1 per cent alcohol solution of rosolic acid in 96 per cent alcohol in proportions of 0.45 c.c. to 5 c.c. Use 5 c.c. of milk and 5.5 c.c. of the reagent.

	Healthy Cows	Just Calved		Mastitis Strepto-coccus	Old Caked Udder
		1 Qt. Caked	Clear		
Average reaction	6.1°	7.75°	7.0°	2.62°	5.45°
Rosolic acid solution	Yellow	Yellow	Yellow	Deep red	Light red
Average Tromsdorff reading	$\frac{5}{16}$	0	$\frac{1}{4}$	Very much	$1\frac{1}{2}$

Because of the gradation of colors from yellow to red it is hard to detect slight changes in reaction by Hoyberg's method. Moreover, the test must be applied soon after milking, because otherwise acidity due to bacterial action overcomes decreased acidity due to inflammation.

b) *Reaction of different grades and classes of milk as received in regular delivery.*—Milk as it is delivered to consumers in our larger cities is from six to 60 hours old. Refrigeration and pasteurization will retard the development of acid-producing organisms.

Based on a large number of observations during the year the average, maximum, and minimum degrees of acidity as milk is received are shown in the following table:

TABLE 9.
SHOWING ACIDITY OF MILK AS IT IS RECEIVED.

	Certified							Inspected		Pasteurized			
	1	7	8	9	10	11	14	4	12	2	3	13	$4\frac{1}{2}$
Average acidity	6.006	5.98	5.95	6.26	6.38	6.37	6.6	6.23	6.41	5.69	5.97	6.36	6.08
Maximum	7.0	7.0	7.0	7.4	7.6	8.0	7.2	8.0	6.8	8.0	7.6	8.2	6.2
Minimum	4.8	5.2	4.4	5.6	5.2	5.2	6.0	5.2	6.0	4.0	5.2	5.2	6.0
Average	Certified 6.18							Inspected 6.306		Pasteurized 5.96			

From Table 9 it will be seen that the lowest degree of acidity was observed in pasteurized milk, and that the same maximum degree observed was found in all three grades. While the variations are marked in all grades and in nearly all supplies, still the entire average for pasteurized market milks was lowest, and for inspected highest, while certified milk stood midway between. This in itself is rather good evidence that the four pasteurized milks are not spoiled milks pasteurized for the purposes of redemption.

B. ACIDITY OF MILK WHEN KEPT FOR VARYING PERIODS OF TIME AT CERTAIN TEMPERATURES.

The changes in acidity which occur in milk when it is kept for a period of time are of more importance than is generally recognized.

TABLE 10.

SHOWING GREATEST VARIATIONS IN ACIDITY BEFORE COAGULATION WHEN MILK IS STORED AT DIFFERENT TEMPERATURES.

Class	When Received	37° C.	Room Temp.		Ice-Box Temp.		1° C.		
		12 Hrs.	24 Hrs.	48 Hrs.	24 Hrs.	72 Hrs.	7 Days	14 Days	25 Days
Certified	4.4 to 8.0	6.4 to 19.6	5.4 to 7.3	5.4 to 14.6	6.0 to 7.4	6.4 to 9.9	5.6 to 7.9	0.8 to 7.6	8.4 to 13.8
Inspected	5.2 to 8.0	16.2 to 27.2	5.9 to 7.0	....	7.2 to 7.5	7.2 to 8.9	5.9 to 7.4	7.0 to 13.4	12.1 to 23.4
2, 3, 13 pasteurized	4.0 to 8.2	20.0 to 24.2	6.8 to 10.4	26.0	5.8 to 13.6	....	6.0 to 8.0	7.0 to 9.8	13.5 to 24.2
4½ pasteurized	6.2 to 6.2	12.2	6.0	....	6.0	6.8	6.2	6.8 to 7.2	7.6 to 11.8

TABLE 11.

SHOWING AVERAGE ACIDITY BEFORE COAGULATION WHEN MILK IS STORED AT DIFFERENT TEMPERATURES.

Class	When Received	37° C.	Room Temp.		Ice-Box Temp.		1° C.		
		12 Hrs.	24 Hrs.	48 Hrs.	24 Hrs.	72 Hrs.	7 Days	14 Days	25 Days
Certified	6.18	11.3	6.4	8.5	6.7	7.7	7.6	6.6	10.6
Inspected	6.306	22.2	6.4	...	7.4	8.1	6.75	8.1	16.3
2, 3, 13 pasteurized	5.96	24.7	9.0	26.0	8.5	...	7.1	8.3	18.3

These changes indicate activity and presence of types of microorganisms and ferments as well as give to the consumer a warning of the unfitness of the milk for household use. One of the objections

to pasteurized milk is that it does not sour or coagulate because the acid-producing organisms have been destroyed by heat, thus removing one of nature's danger signals.

a) Reaction after varying periods of storage at different temperatures before coagulation occurs.—The acidity in milk generally increases on storage, ultimately causing coagulation. To determine the rate of acidification when it is stored at different temperatures, bottles of milk were stored at 37° C., at room temperature, at ice-box temperature, and at a point just above freezing. Examinations were made at regular intervals before and at the time when coagulation occurred. The results of these investigations are shown in Tables 10 and 11.

From these tables it is evident that the acidity of pasteurized milks increases more rapidly and is more marked than that of raw milk. At the higher temperatures the acidity is not only more quickly but more markedly increased than at the lower temperatures.

b) Time required for clotting.—At different times milks were stored at definite temperatures and observations made on the length of time required for coagulation and for changes in reaction.

TABLE 12.

SHOWING WHEN CLOTTING APPEARED ON STORAGE AT DIFFERENT TEMPERATURES.

Temp.	Certified							Inspected		Pasteurized			
	1	7	8	9	10	11	14	4	12	2	3	13	4½
37° C.	36–72 hrs.	24–48 hrs.	12–72 hrs.	24–48 hrs.	24–48 hrs.	48–120 hrs.	12–72 hrs.	12–48 hrs.	24–120 hrs.	12–48 hrs.	12–48 hrs.	12–96 hrs.	12–48 hrs.
Room	3–11 days	3–6 days	2–6 days	2–4 days	3–4 days	3–6 days	3–7 days	2–4 days	2–4 days	3–4 days	2–4 days	2–5 days	1–3 days
Ice-box	5–22 days	6–21 days	6–22 days	6–9 days	6–19 days	7–14+ days	22+–50 days	7–9 days	6–9 days	5–9 days	4–9 days	6–8 days	6–8 days
1° C.	20–37 days	31–46 days	31–39 days	21–34 days	16–66 days	25–53 days	33+–60 days	21–49 days	21–39 days	20–40 days	14–37 days	21–53 days	60+ days

TABLE 13.

SHOWING THE GREATEST DIFFERENCES IN TIME AFTER DELIVERY AT WHICH COAGULATION OCCURRED IN THE DIFFERENT CLASSES OF MILK WHEN KEPT AT DEFINITE TEMPERATURES.

Temp.	Certified	Inspected	Pasteurized
37° C.	12–120 hrs.	12–120 hrs.	12–96 hrs.
Room	2–11 days	2–4 days	1–5 days
Ice-box	5–50 days	6–9 days	4–9 days
1° C.	16–60 days	21–49 days	14–60 days

TABLE 14.

SHOWING THE AVERAGE TIME AT WHICH COAGULATION OCCURS.

Temp.	Certified							Inspected		Pasteurized			
	1	7	8	9	10	11	14	4	12	2	3	13	4½
37° C.	49.3 hrs.	41.14 hrs.	48.8 hrs.	30.8 hrs.	34.3 hrs.	61.7 hrs.	40.8 hrs.	24 hrs.	51.4 hrs.	24 hrs.	23.45 hrs.	42.8 hrs.	24 hrs.
Room	5.0 days	4.16 days	4.85 days	3.14 days	3.25 days	4.57 days	5.75 days	3.125 days	3.0 days	3.125 days	3.0 days	3.5 days	2.75 days
Ice-box	14.6 days	10.7 days	11.7 days	7.37 days	9.1 days	12.4 days	37.0 days	8.125 days	7.57 days	7.25 days	7.125 days	6.87 days	6.75 days
1° C.	32.2 days	36 days	33.75 days	31.3 days	36.4 days	36.5 days	50.0 days	33.8 days	29.5 days	32.14 days	30.4 days	35.0 days	60+ days

TABLE 15.

SHOWING THE AVERAGE TIME REQUIRED AFTER DELIVERY FOR COAGULATION OF THE DIFFERENT GRADES OF MILK AT DIFFERENT TEMPERATURES.

Temp.	Certified	Inspected	Pasteurized
37° C.	44.2 hrs.	36.8 hrs.	28.7 hrs.
Room	4.28 days	3.07 days	3.14 days
Ice-box	11.82 days	7.86 days	6.93 days
1° C.	34.07 days	32.1 days	28.55 days

From Tables 12–15 it is evident that at the temperatures indicated, 37° C., room, ice-box, and just above the freezing point, coagulation occurs in pasteurized milk as soon after delivery as in good raw milks. Moreover, certified milks do not coagulate nearly as soon as inspected and pasteurized milks. At low temperatures coagulation of certified, inspected, and pasteurized milks occurs late, and, as will be shown subsequently, these milks may have a high bacterial content long before they coagulate. Since coagulation is looked upon as the visible means of determining when milk is no longer fresh, these facts are important.

Realizing that if milk is to be kept by the dealer for any length of time before selling the storage temperature must be low, and the

TABLE 16.

SHOWING NUMBER OF DAYS REQUIRED AT 50–60° F. FOR COAGULATION AFTER STORAGE NEAR THE FREEZING POINT.

Days at 1° C.	Certified							Inspected		Pasteurized			
	1	7	8	9	10	11	14	4	12	2	3	13	4½
7 days	6	3	3	4	5	10	10	..	6	6	6	3	4
15 days	11	3	2	4	3	6	15	3	4	3	5	4	4
25 days	1	1	..	1	1	3	3	1	1	1	1	3	9

TABLE 17.

SHOWING MAXIMUM AND MINIMUM TIMES AT 50–60° F. REQUIRED FOR COAGULATION OF MILK PREVIOUSLY STORED FOR VARYING PERIODS OF TIME AT LOW TEMPERATURE.

Days at 1° C.	Certified	Inspected	Pasteurized	Ocean Shipments
7	3–10 days	6 days	3–6 days	5–15 days
15	2–15 days	3–4 days	3–5 days	4–15 days
25	1–3 days	1 day	1–9 days	8–20 days

temperature in the household will at best be that of the ice-box (50–60° F.), an attempt was made to simulate these conditions; the various milks were stored at 1° C. for varying periods of time and then kept at ice-box temperature to determine when clotting occurs.

From Tables 16 and 17 it is evident that milks stored at low temperatures for varying periods of time coagulate more rapidly at the ordinary ice-box temperature than do fresh milks.

c) Reaction when clotting occurs at different temperatures of storage.—On aging, milk no longer consists of cream and skim milk only, but undergoes further separation. Coagulation being due to increased acidity, the reaction of the whole mixture was determined at the time of coagulation (Tables 18–21).

TABLE 18.

SHOWING IN DEGREES REACTION OF WHOLE MIXTURE WHEN COAGULATION OCCURS.

Temp.	Certified							Inspected		Pasteurized			
	1	7	8	9	10	11	14	4	12	2	3	13	4½
37° C.	23.3 to 36.8	22.0 to 48.0	30.0 to 40.0	19.6 to 52.8	24.0 to 46.0	28.0 to 36.0	18.0 to 64.0	27.2 to 89.6	26.0 to 31.8	20.0 to 84.0	24.2 to 76.0	22.4 to 86.0	12.2
Room	28.0 to 42.0	11.0 to 56.0	22.0 to 38.0	24.0 to 38.0	24.0 to 44.0	24.0 to 38.0	10.6 to 28.0	23.8 to 44.0	28.4 to 44.0	26.0 to 60.0	25.6 to 78.0	24.0 to 80.0	6.0
Ice-box	8.0 to 30.0	10.0 to 36.0	24.0 to 28.0	26.0 to 38.0	10.0 to 42.0	18.0 to 33.0	16.0 to 34.6	16.0 to 36.0	24.0 to 38.0	24.0 to 31.7+	24.0 to 38.0	26.0 to 38.0	13.4
1° C.	7.2 to 17.6	25.2 to 34.0	8.0 to 30.0	24.4 to 42.0	20.0 to 40.0	12.0 to 16.6	11.8 to 13.6+	24.0 to 40.0	20.0 to 34.0	26.0 to 40.0	12.0 to 40.0	24.0 to 36.4	...

It is evident from the varying degrees of acidity at which coagulation occurs that acid, at least as determined by titration with NaOH and phenolphthalein, is not the only factor in coagulation. Apparently ferments, peptonization, etc., play some part also.

TABLE 19.

SHOWING GREATEST VARIATIONS IN ACIDITY AT WHICH COAGULATION OCCURRED AT DIFFERENT TEMPERATURES.

Temp.	Certified	Inspected	Pasteurized
37° C.	18.0–52.8	26.0–89.6	20.0–86.0
Room	10.6–56.0	23.8–44.0	6.0–80.0
Ice-box	8.0–42.0	10.0–38.0	13.4–38.0
1° C.	7.2–42.0	20.0–40.0	12.0–40.0

TABLE 20.

SHOWING AVERAGE ACIDITY WHEN COAGULATION OCCURRED AT DIFFERENT TEMPERATURES.

Temp.	Certified							Inspected		Pasteurized			
	1	7	8	9	10	11	14	4	12	2	3	13	4½
37° C.	30.6	37.84	32.5	37.4	35.95	42.85	33.05	50.82	30.95	54.62	45.4	51.28	12.2
Room	34.6	43.75	28.6	31.3	33.95	29.65	20.15	32.45	34.13	39.48	43.9	45.12	6.0
Ice-box	17.1	20.12	25.88	34.72	29.77	25.25	25.3	28.84	28.91	29.0	29.34	30.6	13.4
1° C.	11.2	29.06	19.35	30.06	29.5	14.15	11.8	35.70	26.26	34.76	29.44	30.13	....

TABLE 21.

SHOWING AVERAGE ACIDITY OF DIFFERENT GRADES OF MILK WHEN COAGULATION OCCURS.

Temp.	Certified	Inspected	Pasteurized
37° C.	34.19	44.51	48.77
Room	30.08	33.17	40.31
Ice-box	26.00	28.88	28.78
1° C.	22.85	31.11	31.87

Particular samples of each milk show that the reaction when coagulation occurs varies greatly. The average reaction for all milks at the time of coagulation is highest when kept at 37° C., next highest when kept at room temperature, and lowest at the ice-box temperature except for certified milks. Certified milk coagulates with less acidity than does any other milk, but more time is required, as shown in Table 15.

d) Reaction of different parts of milk physically changed.—Milk, when kept, usually coagulates or clots, and whey becomes separated. These changes are generally due to the production of lactic acid from lactose. Coagulation, however, is not the only physical change observed, for in almost all milks there are also nitrogenous changes which are generally referred to as peptonization and putrefaction. These are by some supposed to make milk alkaline. That this seldom happens will be shown later. What does happen, however, is that milk in which there has been much protein change

is less markedly acid than is other milk. When a yellow fluid forms under the cream it is generally regarded as an evidence of peptonization.

Determinations were made of the reaction of different parts of milk which had undergone coagulation or peptonization.

TABLE 22.

SHOWING AVERAGE REACTION OF DIFFERENT PARTS OF MILK PHYSICALLY CHANGED AS A RESULT OF STORAGE AT DIFFERENT TEMPERATURES.

Storage Temp.	Average Days Kept	Certified			Inspected			Pasteurized		
		Whey	Pep. Fl.*	Mixed	Whey	Pep. Fl.	Mixed	Whey	Pep. Fl.	Mixed
37° C.	7	20.0	29.05	37.4	37.0	33.0	39.3	54.4	51.3	57.3
Room	20	48.0	32.1	42.49	....	32.7	42.7	45.0	46.6	51.46
Ice-box	19	....	22.77	28.6	....	27.3	44.0	....	24.85	36.0
1° C.	34	29.75	13.3	28.19	38.0	30.0	38.0	27.2	28.0	34.6

* Peptonized fluid.

TABLE 23.

SHOWING AVERAGE REACTION OF DIFFERENT PARTS OF CERTIFIED, INSPECTED, AND PASTEURIZED MILKS PHYSICALLY CHANGED AS A RESULT OF STORAGE.

	Whey	Peptonized Fluid	Mixed
Certified	30.54	24.74	33.7
Inspected	37.50	31.33	40.8
Pasteurized	38.62	33.13	40.95
Total average	37.23	28.34	36.44

TABLE 24.

SHOWING AVERAGE REACTION OF DIFFERENT PARTS OF MILKS PHYSICALLY CHANGED AT DEFINITE TEMPERATURES.

Temp.	Whey	Peptonized Fluid	Mixed
37° C.	40.56	33.64	41.05
Room	37.20	30.11	39.98
Ice-box	.....	23.20	31.68
1° C.	30.0	19.6	31.68

From these tables it is evident that the straw-colored (peptonized) fluid which forms under the cream after milk has been stored for some time is less acid than the whey or whole mixture. That the curd contains much acid is shown by the fact that a mixture of the entire quantity is more acid than either the whey or peptonized fluid. This latter conclusion agrees with that of Farrington.[1]

[1] *Wis. Agric. Exp. Sta.*, 1903, *20th Ann. Rept.*, p. 134.

Reaction of cream: Top milk (gravity cream) freshly obtained, or after being stored for several days at a low temperature, is of the same reaction as the skimmed milk below. After storage at ice-box, room, or incubator temperatures for a relatively short period of time, a difference in the acidity of gravity cream and skimmed milk is observed. This is to be expected, for acidity develops only from the serum containing lactose. Farrington emphasizes the fact that the amount of serum present in cream must be taken into consideration when acidity of different samples of cream are compared. To do this he advocates the adoption of the acidity of cream of given richness as a standard, suggesting 0.6 per cent lactic acid in cream containing 25 per cent of fat.

Numerous tests of acidity of gravity cream and skimmed milk were made on delivery of the various milks, and it was found that quite constantly only about five-sevenths as much sodium hydroxid was necessary to neutralize the same amount of cream as of skimmed milk. Acidity determinations of the cream, skimmed and well mixed milks were made after 14 and 25 days of storage at 1° C., and after 14 and 25 days at 1° C. followed by 48 hours of storage at the ice-box temperature. The results are shown in the following table:

TABLE 25.

SHOWING AVERAGE NUMBER OF CUBIC CENTIMETERS OF N/4 NAOH NECESSARY TO NEUTRALIZE 100 C.C. OF CREAM, SKIMMED AND MIXED MILK AFTER STORAGE.

STORAGE	CERTIFIED			INSPECTED			PASTEURIZED (2, 3, 13)		
	Cream	Skimmed	Mixed	Cream	Skimmed	Mixed	Cream	Skimmed	Mixed
14 days at 1° C.	10.3	8.0	8.7	16.8	27.8	25.6	17.1	13.3	14.6
25 days at 1° C.	12.9	9.3	9.3	15.0	18.6	18.8	26.1	38.0	37.0
48 hrs. at 15° C. after 14 days at 1° C.	12.7	10.9	11.5	21.5	77.0	23.6	35.7	36.3	36.8
48 hrs. at 15° C. after 25 days at 1° C.	17.9	25.0	24.6	18.6	28.8	29.8	28.6	46.7	43.4

From this table it will be seen that until there is considerable acidity in the mixed milk the gravity cream is as acid or more acid than the skimmed milk. This holds true of the three classes of milk studied, until the acidity reaches 18°. Whether good milk stored at higher temperatures gives the same reactions I have not determined, nor did I determine whether the results will be the same in separator cream and skim milk stored separately. Aging

of separator cream is resorted to commercially to make cream appear rich.

e) Ultimate changes.—Most milks coagulate naturally with an increase of acidity on storage. Of all the many samples of milks examined only 19 became less acid, and only one became actually alkaline in reaction. Coagulation is, however, not universal, for 42 samples of milk kept at 1° C. did not coagulate after periods of time from one to 10 months. This occurred most frequently in certified and the higher grades of pasteurized milks. In these, changes were evidenced by effervescence when the bottle was opened or by odors indicating protein decomposition. Decomposition without coagulation is of great importance, for by many people milk is considered suitable for human consumption until curdling occurs. While in most cases curdling precedes marked protein changes, still putrefaction may take place and curdling never appear.

Physical changes in milk stored at a definite temperature vary to some extent for different milks, for the same milks, and for different bottles of one supply taken on the same day. In spite of these variations certain changes in physical appearance and reaction may usually be depended on for each grade of milk.

Physical changes: From these changes some of the chemical changes that have occurred may be recognized. The ultimate changes shown in the following table are based on observations made on 380 bottles of milk, of which 64 were stored 220 days at 37° C., 106 for 270 days at room temperature, 86 for 250 days at ice-box temperature, and 124 for 250 days at 1° C.

TABLE 26.

SHOWING PHYSICAL CHANGES OCCURRING IN MILK STORED 220 TO 270 DAYS.

	37° C.			Room			Ice-Box			1° C.		
	Certified	Inspected	Pasteurized	Certified	Inspected	Pasteurized	Certified	Inspected	Pasteurized	Certified	Inspected	Pasteurized
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
No coagulation	0	0	0	0	0	0	6	0	0.0	20	20	7
Solid clot	0	0	0	0	0		0	0	6.5	9	20	23
Clot and clear whey	5	50	0	61	71	22	23	60	32.0	41	40	35
Eroding clot	85	50	100	21	29	61	48	40	65.0	18	20	35
Clot disappearing	10	0	0	18	0	17	23	0	0.0	12	0	0
Putrid without clot	0	0	0	0	0	0	6	0	0.0	20	20	7

From Table 26 it is seen that pasteurized milk is likely to undergo solution of the clot, and that in certified milk complete solution is frequently seen. Coagulation without separation of whey occurs only when milk is refrigerated. Putrefaction without coagulation occurs principally when milks are stored at low temperatures.

When milk is frozen, there is separation into two zones under the cream. Of these, the upper zone is usually translucent, grayish white, while the lower zone is flocculent. A number of bottles of the different supplies received were kept below the freezing point for almost nine months. When these were thawed out the normal appearance was soon re-established, but examination showed that the milks had deteriorated.

Reaction: Inasmuch as the change in reaction of milk is due largely to the action of bacteria, we expect changes of reaction until all food, the metabolism of which influences reaction, is consumed, and until the acid-producing bacteria are destroyed or inhibited in action due to their own products, and the counter-influences on reaction have become stable.

Generally marked acidification is produced if milk is stored a long time, although actual alkalinity or decrease in acidity sometimes is observed. The following table shows the average, maximum, and minimum acidity in degrees for the same 380 bottles stored for periods from 220 to 270 days:

TABLE 27.

SHOWING AVERAGE MAXIMUM AND MINIMUM ACIDITY RESULTING AFTER LONG STORAGE.

	37° C.			Room			Ice-Box			1° C.		
	Certified	Inspected	Pasteurized	Certified	Inspected	Pasteurized	Certified	Inspected	Pasteurized	Certified	Inspected	Pasteurized
Average	93.5	102.3	63.8	78.8	155.7	135.98	60.6	90.5	92.2	33.1	35.8	40.6
Highest	124.8	120.0	109.2	171.2	192.8	203.2	118.4	136.4	159.6	48.0	40.4	54.0
Lowest	Alk.	72.4	3.6	8.0	13.6	56.4	20.0	60.0	55.2	15.0	31.2	29.6

It will be seen from this table that at room temperature the highest acidity is developed, that at 37° C. no alkaline reaction is produced, and that at the lower temperatures acidity is always increased when milk is stored for a long period of time.

Some milks stored at 1° C. for periods varying from six to 10 weeks remained unchanged in appearance. In these the following reactions were observed:

TABLE 28.

SHOWING AVERAGE MAXIMUM AND MINIMUM ACIDITY AFTER SIX TO TEN WEEKS' STORAGE AT 1° C.

	Certified	Inspected	Pasteurized
Average	29.6	33.5	19.9
Highest	51.8	49.8	43.8
Lowest	9.6	24.0	0.8

From this it is seen that pasteurized milk not coagulating on storage at very low temperature acidifies less than raw milk; it also decreases in acidity more frequently during the first months of storage but later becomes as acid as raw milk. When pasteurized milks are kept at room temperature for long periods of time, as is seen from Table 27, they at times become very acid, exceeding the maximum of 0.8 per cent lactic acid, or 35.5°, beyond which Farrington states the acidity will not go.

f) Alkaline milk.—Alkaline milk is frequently referred to, and its occurrence might seem not unusual. Observations of a large number of samples of milk stored for varying periods of time at various temperatures lead me to believe that partial neutralization of the original acidity in fresh milk is infrequent and actually alkaline milk is very rare. While many milks contain organisms which, if acting alone, will produce alkalies, still these milks also contain organisms breaking up the lactose in milk and so there is an increased acidity. Furthermore it has been known for a long time, and has been emphasized more recently by Kendall, that organisms break up the carbohydrates before they act on the proteins, and organisms growing together do not always produce the same end results they produce when grown alone. These mixtures of bacteria in a medium containing the various food materials do not always produce end results representing the sum of their products but rather the products of symbiotic action. Investigations of alkaline milk and of reduction of acidity are shown in Table 29.

This table shows plainly how seldom the acidity of milk is actually decreased on aging and further shows how very rare actual

alkalinity is. It was, however, noticed that if acidity determinations of stored milks are made at frequent intervals, the acidity of some samples at first increases and then gradually decreases. It would seem fair to conclude that in such milks there is alkaline production even though the reaction is still acid.

TABLE 29.

SHOWING OCCURRENCE OF ALKALINE MILK AND REDUCTION OF ACIDITY WHEN MILK IS STORED AT DIFFERENT TEMPERATURES FOR PERIODS VARYING FROM 12 HOURS TO 270 DAYS.

	Alkaline Milk					Milk with Decreased Acidity				
	37° C.	Room	Ice-Box	1° C.	Frozen	37° C.	Room	Ice-Box	1° C.	Frozen
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Certified	3.4	0	0	0	0	0.0	5.5	8.5	7.1	28.5
Inspected	0.0	0	0	0	0	0.0	10.0	0.0	0.0	0.0
Pasteurized, 2, 3, 13	0.0	0	0	0	0	8.5	0.0	0.0	14.7	0.0

g) Odor of milk on aging.—Next to coagulation the odor of milk is the most accessible test of its fitness for use. Fresh clean milk has very little odor, but as changes occur odors and flavors of different kinds result. These odors are of considerable importance in showing the character of changes, and so were noted carefully in a large number of cases. The odors observed when milks coagulate and after long storage are given in Tables 30 and 31.

These tables show that when milks coagulate at 37° C. most of them have a lactic acid odor, and that there is little putrefaction, but after long storage the strong odors of butyric acid and putrefaction become more prominent. At lower temperatures of storage putrefactive odors are not preceded by the usual odor of sour milk

TABLE 30.

SHOWING PERCENTAGE OF OCCURRENCE OF DIFFERENT ODORS AT THE TIME OF COAGULATION.

Temp. of Storage	Certified						Inspected						Pasteurized, 2, 3, 13					
	Acid				Putrid	Fermented	Acid				Putrid	Fermented	Acid				Putrid	Fermented
	Lactic	Acetic	Butyric	Rancid			Lactic	Acetic	Butyric	Rancid			Lactic	Acetic	Butyric	Rancid		
37° C.	68.8	0	7.0	4.9	7.2	11.9	86.6	0	13.4	0	0	0.0	81.6	0	13.6	0.0	4.8	..
Room	43.8	0	31.5	4.9	7.2	12.6	27.3	0	54.6	0	0	18.1	56.4	0	34.7	0.0	4.4	..
Ice-box	4.9	0	26.4	11.9	39.1	17.7	45.4	0	45.4	0	0	9.2	56.0	0	33.3	16.6	16.6	..
1° C.	4.4	0	13.1	17.3	65.2	0.0	0.0	0	44.4	0	0	0.0	7.1	0	14.2	64.5	0.0	..

TABLE 31.

SHOWING PERCENTAGE OF OCCURRENCE OF DIFFERENT ODORS WHEN MILKS ARE STORED FOR PERIODS VARYING FROM 220 TO 270 DAYS.

TEMP. OF STORAGE	CERTIFIED						INSPECTED						PASTEURIZED, 2, 3, 13					
	Acid						Acid						Acid					
	Lactic	Acetic	Butyric	Rancid	Putrid	Fermented	Lactic	Acetic	Butyric	Rancid	Putrid	Fermented	Lactic	Acetic	Butyric	Rancid	Putrid	Fermented
37° C...	0.0	0.0	26.3	26.3	31.6	15.8	20.0	0.0	0.0	40.0	40.0	0.0	0.0	10.0	0.0	10.0	60.0	21.0
Room..	0.0*	16.8	8.4	12.5	41.6	16.8	0.0	37.5	25.0	0.0	12.5	25.0	0.0‡	30.9	15.4	23.1	23.1	0.0
Ice-box	5.2	0.0	15.6	15.6	46.1	17.5	7.2	0.0	14.3	14.3	35.7	28.5	0.0	12.5	0.0	31.2	31.2	25.1
1° C....	10.0	5.0	7.5	7.5	32.5	37.5	†	....	....	....	....	....	14.2	0.0	14.2	42.4	14.6	14.6

* Fatty acid in 3.9 per cent of samples.

† Too few observations to warrant percentage estimates.

‡ Fatty acid odor in 6.6 per cent of samples.

and inasmuch as the detection of these odors when not marked is made only by those properly trained, milks that putrefy without souring are likely to be used when unfit. Putrefaction without souring occurs more frequently in certified than in inspected and pasteurized milks.

3. METHODS FOR READILY DETECTING INCREASED ACIDITY.

Reaction, physical appearance, and odor are of considerable importance as indicators of the fitness of milk as a food. In the home and dairy, physical appearance, clotting on boiling, and odor are relied on; while in the laboratory, determinations of the reaction are added to these indicators. Milk as it is offered for sale in our larger cities is seldom near the souring point as determined by taste and smell, nor have putrefactive changes gone on to the extent of being perceptible by these senses. No milks of which daily deliveries were received at the laboratory had deteriorated sufficiently for one to detect decomposition by taste or smell. Before coagulation occurs increased acidity can be determined by titration in the laboratory. This requires time and for this reason titration cannot be used to prevent the sale of soured milk.

Rapid and convenient methods of detecting increased acidity have been recommended. The use of Farrington's alkaline tablet is one of these, and gives the reaction with phenolphthalein as the indicator. At dairies where milk is received for cheese and butter

making the alizarin test of Engling is used, but it is of value only in the detection of advanced acidity. Rühm has recommended a test for detection of beginning acidification in mixed milks of two or more cows. According to this method 10 c.c. of 68 per cent alcohol are added to 10 c.c. of the milk to be tested. If there is immediate coagulation the acidity is above 8.0°. This test I have tried on a large number of samples and found that the 68 per cent alcohol must be made from absolute alcohol. More advanced acidity is detected by boiling a small amount of milk for a few moments in a test tube. According to Rühm, coagulation on boiling appears if the acidity is above 10.0°.

No milk received at the laboratory coagulated when an equal amount of 68 per cent alcohol was added. The acidity of the various milks as received was from 4.8° to 8.2°. The alcohol and heating methods were also tried on milks stored at different temperatures, the results being shown in Tables 32 and 33.

TABLE 32.

SHOWING VALUE OF 68 PER CENT ALCOHOL AS A MEANS OF DETERMINING BEGINNING ACIDIFICATION, THERE BEING NO COAGULATION ON BOILING.

Temp.	Certified				Inspected				Pasteurized, 2, 3, 13			
	Average Coagulating	Highest Coagulating	Lowest Coagulating	Highest Not Coagulating	Average Coagulating	Highest Coagulating	Lowest Coagulating	Highest Not Coagulating	Average Coagulating	Highest Coagulating	Lowest Coagulating	Highest Not Coagulating
37° C.	9.8	11.1	9.0	...	...	...	...	...	...	...	...	...
Room	8.2	10.6	7.3	7.0	...	...	...	7.0	...	...	8.2	6.0
Ice-box	7.8	9.9	6.8	7.4	8.9	9.8	8.1	7.7	9.0	7.7	8.0	7.2
1° C.	9.5	13.4	7.2	7.6	8.3	8.5	7.9	7.5	8.4	9.8	7.3	7.6

Total average acidity at which coagulation with 68 per cent alcohol occurs equals 8.54°. Highest acidity observed which did not cause coagulation with 68 per cent alcohol equals 7.7°. Lowest acidity observed which caused coagulation with 68 per cent alcohol equals 6.8°.

It will be noticed that by this test lower acidification is detected than by the senses of smell and taste, or by boiling. All samples of milk coagulating on boiling coagulated also on the addition of an equal part of 68 per cent alcohol.

The method most certainly is of value in detecting increase in acidity. Higher percentages of alcohol were tried but were not as satisfactory as the 68 per cent solution.

TABLE 33.

SHOWING VALUE OF BOILING MILK AS A MEANS OF DETERMINING ADVANCED ACIDITY, NORMAL CLOTTING NOT HAVING OCCURRED.

Storage Temp.	Certified				Inspected				Pasteurized			
	Average Coagulating	Highest Coagulating	Lowest Coagulating	Highest Not Coagulating	Average Coagulating	Highest Coagulating	Lowest Coagulating	Highest Not Coagulating	Average Coagulating	Highest Coagulating	Lowest Coagulating	Highest Not Coagulating
37° C........	23.7	37.8	13.8	11.1	21.6	29.2	16.2	...	21.3	28.8	12.2	...
Room	18.1	33.8	11.0	10.6	26.6	28.4	23.8	7.0	15.2	25.6	9.8	8.2
Ice-box......	9.2	10.6	8.4	9.9	....	....	....	...	13.5	13.6	13.2	9.7
1° C.........	10.9	13.8	9.0	13.4	13.4	13.4	11.5	8.5	18.8	11.8	17.2	9.8

Total average acidity at which coagulation on boiling occurs equals 16.2°; highest acidity observed which did not cause coagulation on boiling equals 13.4°; lowest acidity observed which caused coagulation on boiling equals 8.4°.

Acidity and heat are apparently not the only factors in coagulation, as is evident from the variation in acidity when coagulation occurs as a result of boiling. Similar results have been obtained by Stokes,[1] Richmond, and others. Boiling is essentially a home test and by it suspected acidification can be confirmed.

VII. FERMENTS IN MILK.

Ferments or enzymes are usually present in milk. Their importance has been emphasized especially since the introduction of pasteurization. Preservation of enzymes has probably been the most potent factor in reducing the temperature for pasteurization. Many different kinds of enzymes in milk have been described, as galactase, amylase, lipase, peroxidase, catalase, salol-splitting ferments, etc. The original galactase of Babcock and Russell (*loc. cit.*) has been found by Wender[2] to consist of galactase proper, catalase, and peroxidase. Various tests have been devised for detecting the presence of these enzymes. While it is impossible to go into detail in regard to the methods and value of all these tests, a few

[1] *Analyst*, 1900, 16, p. 122. [2] *Oesterr. Chem. Ztschr.*, 1903, 7, p. 1.

are readily made and give much information. Milk ferments are regarded of importance especially in the feeding of infants.[1]

Inasmuch as ferments are produced by bacteria, some discussion has arisen in regard to the origin of the ferments in milk. There now can be no doubt that some ferments may be naturally present in milk, these coming from the cow, while others are produced by the bacteria. To distinguish between and determine the amounts of these ferments tests have been devised, and efforts have been made to ascertain the bacterial content of milk from the amount and activity of its ferments.

The tests used most frequently and successfully were those for catalases and reductases. The following methods were used:

TESTS USED.

CATALASE TEST.

In Lobeck's tube 15 c.c. of the milk to be tested and 5 c.c. of 3 volume per cent hydrogen peroxid were mixed and the stopper tightly inserted. The tube for measuring the liberated oxygen was then filled with water and inserted in the hole in the stopper, pushing out the small hard rubber button. The mixture of milk and hydrogen peroxid was immersed up to the stopper in a water bath at 37° C. and left there for two hours. The oxygen liberated replaced the water in the graduated tube after which the readings were made. The method is similar to that devised by Rühm and Auzinger, except that they used 10 c.c. each of milk and hydrogen peroxid. I used larger quantities of milk and less hydrogen peroxid so as to get satisfactory readings for pasteurized milk. According to Auzinger much gas occurs (1) with physiologically changed milk as is the case with colostrum and with milk from old milkers, (2) with pathologically changed milk as in mastitis and other febrile diseases, or (3) with bacteria rich milk.

REDUCTASE TESTS.

a) Schmidt-Müller or slow reductase test.—The reagent is made by adding 195 c.c. of distilled water to 5 c.c. of saturated alcoholic solution of methylene blue (zinc chlorid double salt). This reagent should be boiled every day before using. The test is made by adding to 20 c.c. of milk in a test tube 1 c.c. of the reagent,

[1] Freeman, *Jour. Am. Med. Assoc.*, 1907, 49, p. 1740.

mixing, sealing with melted paraffin, and then incubating at 45° C. in a water bath. According to Rühm fresh milk remains blue for 12 hours or more, and infected milk decolorizes in less than one hour. Reductases, according to Rühm, are increased by acid formers but not by alkaline producers. Auzinger, quoting from Jensen, who uses 0.5 c.c. of the reagent in 20 c.c. of milk, states that on holding the mixture at 38° to 40° C. milk not decolorizing in seven hours contains less than 100,000 bacteria per cubic centimeter, that which decolorizes in two to seven hours contains 100,000 to 300,000, and that which decolorizes in one-fourth to two hours contains 300,000 to 20,000,000 bacteria per cubic centimeter.

b) Schardinger or hastened reductase test.—The reagent is made by adding 190 c.c. of distilled water and 5 c.c. of formaldehyde solution to 5 c.c. of saturated alcoholic solution of methylene blue (zinc chlorid double salt). The test is made by adding to 10 c.c. of milk 2 c.c. of the reagent, mixing well, sealing with melted paraffin, and holding at 37° C. in a water bath. By the test, according to Auzinger, good milk reduces the color in eight to twelve minutes, milk rich in bacteria reduces in five minutes or less, and when colostrum is present two or more hours are required.

Of the two reductase tests, according to Schardinger, reduction by the slow method is due to ferments produced by bacteria, while by the hastened method reduction is due to the natural ferments of milk.

STORCH TEST.

This test is made by adding to 5 c.c. of milk one drop of 0.2 per cent H_2O_2, and two drops of a 2 per cent solution of paraphenylendiamin and thoroughly mixing. The reagent must be freshly made at least every two weeks.

WILKINSON AND PETERS TEST.[1]

This test is made by adding to 10 c.c. of milk 2 c.c. of a 4 per cent alcoholic benzidine solution and two or three drops of acetic acid, then mixing well and adding 2 c.c. of 3 per cent H_2O_2.

GUIAC TEST.

The reagent is made by adding one part of guiac to ten parts of acetone. To make the test several drops of 0.2 per cent H_2O_2

[1] *Ztschr. Nahr. u. Genussm.*, 1908, 16, p. 172.

and 1 c.c. of the guiac solution are added to 10 c.c. of milk. The reaction appears in one to three minutes.

BELLEI TEST.[1]

The test is made by adding to 10 c.c. of milk three drops of 1.5 per cent aqueous solution of ortol and two drops of 3 per cent H_2O_2.

The Storch, Wilkinson and Peters, guiac, and Bellei tests are used primarily to detect heating above 70° C. and are of little value with us, as heating to such high temperatures is seldom resorted to in this country.

RESULTS OBTAINED

The results obtained with ferment tests are shown in Tables 34-37.

TABLE 34.

SHOWING AVERAGE, HIGHEST, AND LOWEST CATALASE DETERMINATIONS OBSERVED IN MILKS AT THE TIME OF DELIVERY, AND HIGHEST OBSERVATIONS MADE BEFORE COAGULATION OCCURRED AT THE VARIOUS TEMPERATURES OF STORAGE.

Supply	No.	As Received			After Storage			
		Average	Highest	Lowest	37° C.	Room Temp.	Ice-Box Temp.	1° C.
Certified	1	2.53	3.4	1.4	6.4	4.2	4.4	15
	7	2.52	4.2	0.8	3.0	7.4	15.0	20
	8	2.26	2.4	1.2	4.2	8.2	15.0	15
	9	2.0	2.6	1.2	4.2	7.8	15.0	15
	10	1.92	2.8	1.4	4.0	7.8	8.0	10
	11	1.63	2.9	1.2	3.8	2.0	5.8	15
	14	1.8	2.2	1.0	15.0	4.0	15.0	20
Insp.	4	3.06	4.0	2.4	3.2	4.4	3.6	20
	12	2.78	4.8	1.2	3.4	4.6	7.6	15
Pasteurized	2	1.23	1.6	0.2	4.4	4.4	15.0	20
	3	1.7	2.5	0.6	2.6	5.2	5.6	20
	13	1.7	2.5	0.8	2.8	4.6	10.4	15
	4½	0.98	1.4	0.6	1.0	1.6	5.4	2

TABLE 35.

SHOWING AVERAGE CATALASE DETERMINATIONS IN MILKS AS RECEIVED AND AVERAGE OF HIGHEST OBSERVATIONS BEFORE COAGULATION OCCURRED AT THE VARIOUS TEMPERATURES OF STORAGE.

	As Received			After Storage			
	Highest	Lowest	Average	37° C.	Room Temp.	Ice-Box Temp.	1° C.
Certified	4.2	0.8	2.1	6.51	5.91	14.03	18.5
Inspected	4.8	1.2	2.92	3.3	4.5	5.6	20.0
Pasteurized, 2, 3, 13	2.5	0.2	1.54	2.45	4.7	12.0	22.0

[1] *Centralbl. f. Bakt. u. Parasit.*, 1904, 12, p. 518.

Raw milks liberate more oxygen from hydrogen peroxid than do pasteurized milks. At the higher temperatures of storage catalases are not as much increased up to the time of coagulation as they are when milks coagulate at the lower temperatures. The tests are of value when milk ages at the temperatures usually met with in the milk trade and any reading above five should suggest further investigation.

TABLE 36.

SHOWING RESULTS OF REDUCTASE TESTS AND BACTERIAL COUNTS MADE ON THE MILKS WHEN RECEIVED.

Supply	No.	Slow Reductase (hours)		Hastened Reductase (minutes)			Bacteria per c.c.		
		Fastest	Slowest*	Average	Fastest	Slowest*	Average	Highest	Lowest
Certified	1	1:00	60%−	9.1	5	17	3,756	10,500	800
	7	....	100%−	8.3	7	19	6,650	27,000	1,800
	8	1:30	43%−	....	9	10%−	8,935	24,000	1,400
	9	....	100%−	....	9	30%−	20,650	51,000	3,500
	10	....	100%−	....	12	10%−	13,900	52,000	900
	11	....	100%−	10.7	7	22	3,543	14,000	600
	14	5:00	80%−	9.4	12	4	3,470	7,100	800
Insp.	4	2:00	40%−	10.1	8	13	33,610	52,000	12,500
	12	2:30	80%−	13.1	8	44	11,545	99,000	1,300
Pasteurized	2	4:30	50%−	....	9	60%−	310,250	840,000	6,000
	3	3:00	30%	....	110	80%−	285,750	1,640,000	16,000
	13	12:00	60%	....	25	40%−	91,600	1,569,000	4,000
	4½	....	100%	....	20	80%−	4,020	9,700	200

* The figures in these columns show the longest time taken to decolorize the specimens. The percentage figures show what percentage of specimens were negative or failed to decolorize.

Examination of Table 36 shows plainly that reductases must be of two kinds, those naturally present in milk and those produced by the bacteria. The slow reductase test is not entirely reliable for the determination of numbers of bacteria present, as is seen by comparing the observations on supplies 1 and 8 with supplies 9 and 10. All bacteria do not produce reductases which can be tested for by the Schmidt-Müller test, for pasteurized milks at times showed high bacterial counts and still such milk did not reduce at all or only slowly. When milk is coagulated the tests are no longer satisfactory.

These tests were tried on milks that had been stored at the different temperatures. Generally with the increased bacterial count there was an *accelerated* reduction with the Schmidt-Müller test, and while this test is not absolute, decolorization in less than

one hour is practically always an evidence of a high bacterial content, and decolorization in less than two hours should indicate further investigation.

The hastened or Schardinger reductase test is of great value, not as an indicator of bacterial content, but for the detection of heating of milk. Milk heated to 140° F. or above, while exposed to the air, generally will not reduce by this test, and when milk pasteurized in bulk reduces it is evidence of inefficient pasteurization. Likewise *mixed* milk that will not reduce has been heated

TABLE 37.

SHOWING CONCENTRATION OF FERMENTS IN GRAVITY CREAM, WHEN MILKS WERE STORED AT 1° C. FOR 14 DAYS.

Supply	No.	Catalase			Hastened Reductase (minutes)			Slow Reductase (minutes)		
		Cream	Skimmed	Mixed	Cream	Skimmed	Mixed	Cream	Skimmed	Mixed
Certified	1	40.0	3.0	6.0	1	9	5	3	Neg.	4
	7	50.0	15.0	20.0	1	4	2	7	20	10
	9	60.0	1.8	11.2	2	15	5	3	Neg.	70
	10	50.0	4.2	30.0	3	15	6	4	318	30
	11	15.0	1.2	2.4	1	24	11	2	120	2
	14	20.0	1.8	9.2	5	Neg.	20	1	7	4
Insp.	4	15.0	3.2	5.6	20	40	30	5	10	4
	12	60.0	3.8	5.2	2	15	5	2	Neg.	11
Pasteurized	2	40.0	1.6	2.0	3	6	4	6	19	16
	3	16.2	4.0	3.0	20	40	30	Coag.	Coag.	Coag.
	13	40.0	4.0	5.2	10	40	20	8	Neg.	20
	4½	6.0	0.0	0.8	3	Neg.	Neg.	30	Neg.	Neg.

to 140° F. or above. Here, however, it must be remembered that milk from one cow may not contain reductases detectable by this test. In two different herds a number of cows were found whose milk did not reduce. Heating in the sealed bottle does not destroy reductases as markedly as does heating when milk is exposed to the air.

Storch, Wilkinson and Peters, guiac, and Bellei tests were applied throughout the entire year. These tests are of little value unless milk has been heated beyond 70° C. Only a few bottles of pasteurized milk showed overheating during the entire time milks were received at the laboratory.

Babcock and Russell (*loc. cit.*) in 1897 in connection with experiments on the ripening of skimmed milk and full cream cheeses pointed out that in any creaming process the natural enzymes of milk are separated largely from the skimmed milk. In my investi-

gation on gravity cream and skimmed milk the appearance of ferments in gravity cream was noted. Table 37 shows some of the results obtained.

This table shows that gravity cream contains larger amounts or more active ferments than does the skimmed milk. Even in pasteurized milk the ferments in cream are considerable. Whether separator cream contains relatively more ferments than the milk from which it is obtained I have not determined. Babcock and Russell's experiments show that much galactase is lost in separator slime. The importance of ferment concentration in gravity cream may be considerable in infant feeding.

VIII. SUMMARY AND CONCLUSIONS.

Milk from the better class of dairies in large cities is generally delivered in neat and clean bottles. Good milk and good service are so important to the consumers that the drivers of milk wagons soon become interested in furnishing good milk to their family trade.

Tests that give results readily and quickly are most important because a new supply of milk must be available daily. It is of little value to the consumer to learn that the milk he used yesterday or a week ago was unfit.

Our better classes of milk contain little sediment, market milks containing more dirt but fewer cells than certified milks. The Tromsdorff tube is a convenient method for examining for dirt and cells.

It is relatively easy to produce milk of low bacterial content but the greatest care must be constantly exercised to prevent occasional high counts.

Increase in bacterial content was more rapid at the higher temperatures, and at a temperature just above freezing there was little bacterial multiplication until after the seventh day, but from that time on the increase was rapid. Cream on rising carries with it a large portion of the bacteria in milk. Separator cream does not take with it as large a portion of the bacteria as does gravity cream. When milk was stored and the cream and skimmed milk were not remixed, bacterial multiplication was not as great during the same period of storage as when the milk was mixed repeatedly.

Of the bacteria in good milks about 30 per cent were acid-producers, while 20 per cent of those in inspected and 30 per cent of those in certified and pasteurized milks were protein-digesters. At the higher temperatures fermenting organisms increased more rapidly in certified milk, but at the lower temperatures the most marked increase was observed in pasteurized milk. The percentage of peptonizing types increased only at low temperatures. Apparently gravity cream contains the larger portion of peptonizing forms, while the skimmed milk below contains most of the fermenting forms when milk has been refrigerated.

In a lactose medium 41.43 per cent of certified, 55 per cent of inspected, and 84 per cent of pasteurized milks produced gas. Certified milk did not contain bacteria producing hydrogen sulfid and indol as frequently as did inspected and pasteurized market milks.

Milk from definitely diseased quarters is less acid than from healthy quarters. At the time of delivery the acidity of milks varied markedly, the average being lowest for pasteurized milks and highest for inspected milk. The acidity in all milks increased more markedly at the higher temperatures of storage, and at all temperatures the increase was greatest in pasteurized milk.

Coagulation occurred as soon after delivery in pasteurized market milk as in raw milks. Milk stored at a temperature just above the freezing point showed marked bacterial increase before coagulation occurred. The acidity of milk at which coagulation occurred varied. It was highest after storage at 37° C. and room temperature, and lowest when milk was stored in the ice-box. Certified milk coagulated at a lower acidity than did the other grades of milk but it took longer to get the coagulating acidity in certified milk. Alkaline milk was very infrequent.

When clotting had occurred the curd was most acid. The straw-colored fluid under the cream is an evidence of peptonization and was not as acid in reaction as the curd and whey. Gravity cream, above the skimmed milk, was as acid as the skimmed milk as long as the acidity of the mixed milk was not high.

On long storage butyric acid and putrefactive odors became marked. When milk was stored at low temperatures the develop-

ment of putrefactive odors may not have been preceded by acid odors. If milk is stored for a long time the protein of certified and pasteurized milks may be entirely broken down.

Protein decomposition without coagulation occurred principally at a temperature near the freezing point.

Adding an equal part of 68 per cent alcohol to milk is an easy and reliable test for the detection of beginning acidification.

The tests for catalases and reductases are of much value. The catalase and slow reductase tests are of assistance in detecting old milk, and the hastened reductase test offers a convenient and reliable method for detecting and testing the efficiency of pasteurization. Gravity cream carries with it a large portion of the ferments of milk.

The fear of putrefying organisms in pasteurized milk is not warranted as far as market milk pasteurized by the holding method is concerned. While predominance of putrefying bacteria is not the only objection to pasteurized milk, it has been an important one. Certified milk, because it contains but little cow manure, is infected principally with spore-bearing organisms, it is always well refrigerated, and contains as large a percentage of protein-digesting and no more acid-forming bacteria than does pasteurized milk.

The cleanliness necessary to produce certified milk is so imperative and the variation in the production and pasteurization of ordinary market milk so marked, that certified and pasteurized milks are most variable. We find therefore such great variations in numbers and kinds of bacteria in certified and pasteurized market milk that the bacterial content cannot be foretold, the best information being that based on a large number of observations of a number of supplies of these grades of milk. Inspected milk is apparently more uniform in numbers and kinds of bacteria.

These investigations were made possible by the establishment of a milk research fellowship in the Department of Preventive Medicine and Hygiene of the Harvard Medical School by the Milk and Baby Hygiene Association of Boston. I wish here to acknowledge my appreciation of the interest and valuable suggestions and assistance of Professor M. J. Rosenau, as well as to express my thanks to the Milk and Baby Hygiene Association for giving me the opportunity to make these investigations and to the various producers and distributers of milk in Boston and vicinity who so kindly supplied me with the milk used.